



Faculty of Resource Science and Technology

**GENE EXPRESSION ANALYSIS OF SELECTED
RIBOSOMAL PROTEIN GENES IN
NASOPHARYNGEAL CELLS**

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Bachelor of Science with Honours

(Resource Biotechnology)

2008

**Gene Expression Analysis of Selected Ribosomal Protein Genes in
Nasopharyngeal Cells**

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**A Thesis submitted in partial fulfillment of the requirement for the degree
of Bachelor of Science with Honours (Resource Biotechnology)**

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2008

ACKNOWLEDGEMENTS

First of all, I would like to express my highest thanks and deepest appreciation to my supervisor, Dr Edmund Sim Ui Hang for his excellent supervision and guidance throughout the entire duration of my project. I am very grateful that he has imparted me with many invaluable knowledge and experiences in this project. In addition, I would also like to grab this opportunity to express my utmost thanks and gratefulness to Dr. Awang Ahmad Sallehin for his timely advice and helpful guidance in protein study.

Apart from that, I would also like to express my sincere gratitude to the postgraduate students of the Human Molecular Genetics Laboratory, UNIMAS – Ma Xiang Ru, Chia Sze Wooi, Nur Diana Anuar, Chua Suk Ngo, Johnson Chong and Ang Chow Hiang - for their invaluable advice and generous help throughout the whole duration in completing my project. Besides, I would like to dedicate my special thankfulness to Ang Chun Huap from Molecular Genetic laboratory in assisting me the protein works. Not forgetting, the laboratory assistants - Miss Limjantai Kadin Patrick and Mr Ajis Ajim - for their generous assistance in the laboratory.

Last but not least, I would like to express my special gratitude to my parents for their moral support and encouragement since the commencing of this project. Besides, I would also like to extend my thankfulness and appreciation to my coursemates and juniors for their generous support, care and concern during the course of the project.

TABLE OF CONTENTS

Acknowledgements	i
List of Tables	v
List of Figures	vi
List of Abbreviations	vii
Abstract	xii
Abstrak	xii
Chapter 1 Introduction	1
Chapter 2 Literature Review	
2.1 Nasopharyngeal Carcinoma (NPC)	4
2.2 Ribosomal Proteins (RPs)	9
2.3 Implication of Ribosomal Proteins Defects in Human Diseases	12
2.4 Regulatory Mechanism of Gene Expression of Ribosomal Proteins	16
Chapter 3 Materials and Methods	
3.1 Cell Cultures	18
3.2 mRNA Expression Analysis	
3.2.1 Total RNA Extraction from Cell Lines	18
3.2.2 Agarose Gel Electrophoresis (AGE)	20

3.2.3	Quantitation of RNA samples	20
3.2.4	Reverse Transcription (RT)	20
3.2.5	Polymerase Chain Reaction (PCR)	22
3.2.6	Agarose Gel Electrophoresis (AGE)	24
3.2.7	Expression Analysis	25
3.3	Protein Expression Analysis	
3.3.1	Preparation of Lysis Buffer	25
3.3.2	Preparation of Cell Lysates from Cell Lines	26
3.3.3	Denaturation of Proteins	26
3.3.4	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	26
3.3.5	Western Blotting (WB)	27
Chapter 4	Results	
4.1	Cell Cultures	30
4.2	Total RNA Extraction from Cell Lines	31
4.3	Polymerase Chain Reaction (PCR)	32
4.4	mRNA Expression Analysis	35
Chapter 5	Discussions	41
Chapter 6	Conclusions and Recommendations	44

Chapter 7	References	46
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Appendices

Appendix A	Second Report of National Cancer Registry	52
Appendix B	PCR Optimization Attempts for <i>RPS26</i> gene	54
Appendix C	PCR Optimization Attempts for <i>RPS27</i> gene	56

LIST OF TABLES

Table 1	Contents of a 20 µl RT mixtures	21
Table 2	Contents of a 25 µl PCR mixtures using <i>GoTaq</i> [®] DNA polymerase	23
Table 3	Contents of a 25 µl PCR mixtures using DNA <i>Taq</i> polymerase	23
Table 4	Full length cDNA primer sets used to amplify <i>GAPDH</i> , <i>RPS26</i> and <i>RPS27</i> genes	24
Table 5	The band density values obtained from the amplification of <i>GAPDH</i> , <i>RPS26</i> and <i>RPS27</i> genes on normal nasopharyngeal carcinoma, NPC tissues and NPC cell lines	35
Table 6	t-test showing the significance of gene expression for <i>RPS26</i> and <i>RPS27</i> between normal nasopharyngeal tissue (sample H81) and NPC tissues (sample H96)	37
Table 7	t-test showing the significance of gene expression for <i>RPS26</i> and <i>RPS27</i> between normal nasopharyngeal tissue (sample H81) and NPC cell lines (HONE1, SUNE1 and TWO1)	38
Table 8	One-way ANOVA showing the significance of gene expression for <i>RPS26</i> and <i>RPS27</i> among three NPC cell lines (HONE1, SUNE1 and TWO1)	39
Table 9	Parameters used in PCR attempts in optimizing annealing temperature and condition for <i>RPS26</i> for samples H81, H96, HONE1, TWO1 and SUNE1.	54
Table 10	Parameters used in PCR attempts in optimizing annealing temperature and condition for <i>RPS27</i> for samples H81, H96, HONE1, TWO1 and SUNE1.	56

LIST OF FIGURES

Figure 1	Location of nasopharynx	6
Figure 2	Pathogenesis model of NPC	8
Figure 3	Flow chart of gene expression analysis	29
Figure 4	The growth conditions of cell lines. a) The 8 th passage of HONE1 cell line. b) The 9 th passage of TWO1 cell line. c) The 5 th passage of SUNE1 cell line.	30
Figure 5	Agarose gel electrophoresis of RNA extraction from cell lines	31
Figure 6	Agarose gel electrophoresis of PCR amplification of <i>GAPDH</i> on normal nasopharyngeal mucosa, NPC tissues and NPC cell lines	32
Figure 7	Agarose gel electrophoresis of PCR amplification of <i>RPS26</i> on normal nasopharyngeal mucosa, NPC tissues and NPC cell lines	33
Figure 8	Agarose gel electrophoresis of PCR amplification of <i>RPS27</i> on normal nasopharyngeal mucosa, NPC tissues and NPC cell lines	34

LIST OF ABBREVIATIONS

~	approximate
<	less than
%	percentage
⁰ C	degree Celcius
µg	microgram
µl	microlitre
AGE	agarose gel electrophoresis
AMV	Avian Myeloblastosis Virus
ANOVA	Analysis of Variance
bp	basepair
BSA	bovine serum albumin
cDNA	complementary deoxyribonucleic acid
cm	centimeter
CO ₂	carbon dioxide
Conc.	concentration
df	degree of freedom
dT	deoxythymidine
Da	Dalton
DEPC	diethyl pyrocarbonate
DNA	deoxyribonucleic acid

dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
dNTP	deoxyribonucleotide triphosphate
EBV	Epstein-Barr virus
EDTA	ethylenediamine tetra-acetic acid
EST	expressed sequence tag
EtBr	ethidium bromide
g	gram
F	forward
FBS	fetal bovine serum
GAPDH	glyceraldehydes-3-phosphate dehydrogenase
GLD	gel loading dye
HCl	hydrogen chloride
HEPA	high-efficiency particulate air
HLA	human leukocyte antigen
H ₂ O	water
kDa	kilo Dalton
L	large
LMP 1	latent membrane protein 1
M	molar

MgCl ₂	magnesium chloride
Min	minutes
mg	milligram
MgCl ₂	magnesium chloride
ml	millilitres
mM	milimolar
M-MLV	Moloney Murine Leukimia Virus
MPS-1	metallopanstimulin-1
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
nm	nanometer
NaOH	sodium hydroxide
NP-40	Nonident P-40
NPC	nasopharyngeal carcinoma
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pmol	picomolar
PMSF	Phenylmethylsulfonyl flouride
PVDF	polyvinylidene difluoride
R	reverse
RNA	ribonucleic acid
RNase	ribonuclease

RP	ribosomal protein
rRNA	ribosomal ribonucleic acid
rpm	revolution per minutes
RPMI	Roswell Park Memorial Institute
RT	reverse transcription
RT-PCR	reverse transcription polymerase chain reaction
S	small
SCC	squamous cell carcinoma
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
Sec	seconds
T-flask	Tissue culture flask
TAE	tris acetate EDTA
Temp.	temperature
Tris	tris (hydroxymethyl) methylamine
U	unit
μl	microliter
μg	microgram
UV	ultraviolet
v	volume
V	volts
w	weight

WB	Western blotting
WHO	World Health Organization

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ABSTRACT

Ribosomal proteins (RPs) are the essential constituents of ribosomes in all organisms. The investigations, to date, have reported the implication of ribosomal proteins in tumourigenesis. Recently, a preliminary study revealed the down-regulation of *RPS26* and *RPS27* genes in nasopharyngeal carcinoma (NPC) relative to normal nasopharyngeal tissues. Hence, it is essential to study the gene expression of both *RPS26* and *RPS27* in NPC in order to obtain an insight into the actual roles and precise mechanism of these two ribosomal proteins in tumourigenesis of NPC. This project aimed to evaluate the gene expression patterns of both *RPS26* and *RPS27* in NPC at transcript and protein level using comparative reverse-transcription polymerase chain reaction (RT-PCR) and Western analysis. In this study, total RNA isolated from normal nasopharyngeal mucosa tissues, NPC tissues and NPC cell lines were successfully amplified with RT-PCR. Specific bands of PCR products with estimated size of 572 bp and 300 bp were obtained for *RPS26* and *RPS27* genes respectively. *RPS26* mRNAs was found to be up-regulated in NPC cell lines and tissues relative to normal nasopharyngeal mucosa. However, there is no significant difference for *RPS26* and *RPS27* mRNA expression in both NPC tissues and NPC cell lines relative to the normal nasopharyngeal mucosa. Moreover, no significant variation was observed for *RPS26* and *RPS27* mRNA expression among three different cell lines.

Keywords: *RPS26*, *RPS27*, RT-PCR, Western analysis, NPC

ABSTRAK

Bagi semua organisma, protein-protein ribosoma (RPs) merupakan komponen penting dalam pembentukan ribosoma. Sehingga kini, beberapa kajian telah melaporkan penglibatan protein-protein ribosoma dalam perkembangan kanser. Kebelakangan ini, satu kajian awal telah mendapati bahawa gen *RPS26* dan gen *RPS27* mengalami penurunan pengekspresan dalam karsinoma nasofaringeal berbanding dengan tisu nasofaringeal yang normal. Maka, kajian pengekspresan gen bagi *RPS26* dan *RPS27* adalah sangat penting untuk mendapat satu petunjuk terhadap peranan dan mekanisme sebenar mereka dalam perkembangan NPC. Projek ini bertujuan untuk menilai pengekspresan gen bagi *RPS26* and *RPS27* dalam NPC pada peringkat transkrip dan protein dengan menggunakan RT-PCR dan analisis Western. Dalam kajian ini, keseluruhan RNA yang dipencilkan daripada tisu nasofaringeal yang normal, tisu NPC dan sel-sel temurun NPC telah berjaya diampifikasikan melalui RT-PCR. Jalur spesifik bagi *RPS26* dan *RPS27* gen dengan size anggaran sebanyak 572 bp dan 300 bp masing-masing telah diperolehi. mRNA bagi *RPS26* telah menunjukkan pengekspresan keterlaluan dalam tisu NPC dan sel-sel temurun NPC berbanding dengan tisu nasofaringeal yang normal. Namun, *RPS27* menunjukkan pengekspresan yang sama dalam semua tisu nasofaringeal yang normal, sel-sel temurun NPC dan tisu NPC. Selain itu, mRNA bagi kedua-dua *RPS26* dan *RPS27*gen tidak menunjukkan sebarang pembezaan dari segi pengekspresan antara sel-sel temurun NPC.

Kata kunci: *RPS26*, *RPS27*, RT-PCR, analisis Western, NPC

CHAPTER 1

INTRODUCTION

According to Kenmochi *et al.* (2000), the effects of ribosomal mutation and their roles in human diseases have been poorly explored. It is not yet fully understood how mutation in ribosomal protein (RP) genes occur and how it might lead to specific cellular changes. However, the emergence of evolutionary and genetic studies leads the investigators to predict the association of ribosomal mutation with human congenital disorders (Kenmochi *et al.*, 1998). Few previous studies have demonstrated the correlation of RP genes deficiency with human diseases. For instance, mutant *RPL6* gene was associated with Noonan Syndrome (Kenmochi *et al.*, 2000), *RPS4* deficiency was observed in Turner Syndrome (Kenmochi, *et al.*, 1998) and *RPS19* gene has been mutated in patients with Diamond-Blackfan anaemia (Draptchinskaia *et al.*, 1999; Willig *et al.*, 1999). Kenmochi *et al.* (1998) has suggested that any quantitative deficiency in ribosomal protein genes will result in reduced protein synthesis and thereby yield individuals with specific, abnormal phenotype. Furthermore, there are some evidences to show that the expression level of ribosomal protein genes is closely associated with neoplasia. A study conducted by Pogue-Geile *et al.* (1991) has revealed the up-regulation of *RPS3*, *RPS6*, *RPS8* and *RPS12* mRNA in both colorectal carcinoma and polyps. Moreover, Wang *et al.* (2000) has found that the *RPL7a* was up-regulated in colorectal carcinoma. Thus, the findings have revealed that the role of ribosomal protein genes in tumourigenesis can no longer be ignored.

Recently, a preliminary study (Sim, E. unpublished data) has reported that two ribosomal protein genes, *RPS26* and *RPS27* are over-expressed in normal nasopharyngeal mucosa relative to those of nasopharyngeal carcinoma (NPC). The up-regulation of both *RPS26* and *RPS27* mRNA in normal nasopharyngeal tissues was demonstrated by modified RT-PCR differential display assay (Gene FishingTM Technique). This preliminary discovery provides an insight into the involvement of *RPS26* and *RPS27* genes in tumourigenesis of NPC. Previous studies reported the involvement of *RPS27* in tumourigenesis of prostatic carcinoma (Fernandez-Pol, *et al.*, 1997) as well as in regeneration and oncogenesis of hepatocellular carcinoma (Ganger *et al.*, 2001). Moreover, *RPS27* has been found to be differentially expressed in normal breast tissues and tumour tissues (Atsuta *et al.*, 2002). However, no established information is currently available regarding the implication of *RPS26* in cancer progression.

To date, the actual role and precise mechanism of ribosomal proteins in progression of cancer remain largely unclear. Furthermore, there is no established data that correlates the RP mutation to NPC. The identification of *RPS26* and *RPS27* genes in preliminary finding (unpublished report) only gives the first clues on the implication of these genes in NPC. The complete genetic mechanism of these two genes in NPC development still remains unknown. In order to obtain a better understanding on biochemical roles and molecular mechanisms of *RPS26* and *RPS27* in tumourigenesis of NPC, we looked for the differential gene expression of *RPS26* and *RPS27* genes in NPC cell lines and normal nasopharyngeal tissues. The gene expression patterns of *RPS26* and *RPS27* genes may serve as a unique characteristic of NPC.

Hence, this project aims to evaluate the gene expression patterns of *RPS26* and *RPS27* in different cell lines at both the transcript and protein level.

In this project, total RNAs were isolated from HONE1, TWO1 and SUNE1 cell lines and amplified with reverse-transcription polymerase chain reaction (RT-PCR). Then, the mRNA expression levels in different cell lines, NPC tissues as well as the normal nasopharyngeal mucosa were analyzed by Alpha Ease FC software (Alpha Innotech, California) and compared to detect for any variations present. For further validation, total proteins were extracted from those three types of cell lines and subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

CHAPTER 2

LITERATURE REVIEW

2.1 Nasopharyngeal Carcinoma (NPC)

Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma arising from the epithelium that covers the surface of nasopharynx (Brennan, 2006). Most of the NPC originates from the fossa of Rosenmuller, often in the recess (Tao & Chan, 2007).

NPC is a rare cancer disease in most part of the world. It has remarkably distinct racial and geographic distribution. The highest incidence is observed in Southern China and Southeast Asia. In Southern China, NPC occurs in 30 per 100000 persons annually (Tao & Chan, 2007). The incidence rate is 100-fold higher than that in Caucasians (Chi, 2007). In USA and Canada, the annual incidence rate is < 1 per 100000 persons annually, accounting for 0.2% of all cancer incidences (Tao & Chan, 2007). In addition, men are twice likely compared to women in developing NPC. In Malaysia, NPC is the second most common cancer among men after lung cancer by constituting 8.8% of total male cancers (Lim & Halimah, 2004). In 2003, the Malaysian Chinese men had the highest age-standardized incidence rate of 18.1 per 10000 persons annually (Lim & Halimah, 2004).

The nasopharynx is a box-like chamber that lies at the back of nose toward the skull base and above the soft palate (roof of the mouth). The nasopharynx consists of several types of tissues; each of them contains different types of cells. The World Health Organization (WHO) has classified NPC into three histopathological types based on the degree of differentiations (Kwok, *et al.*, 2004). WHO Type I is keratinizing squamous cell carcinoma (SCC) which is characterized by well-differentiated cells that produce keratin. SCC also consists of intracellular bridges similar to typical squamous cell carcinoma. WHO Type I is prevalent in Western countries as it accounts for more than 75% of the NPC incidences (Marks *et al.*, 1998). WHO Type II is non-keratinizing carcinoma in which the cells varied from mature to anaplastic in morphology and produced minimal amount of keratin. It only represents 12% of NPC in North America and ~3% in Southern China (Tao & Chan, 2007). WHO Type III is undifferentiated carcinoma which has typical morphology with a prominent lymphoplasmacytic infiltrate. This type of carcinoma is referred as “lymphoepithelioma”. More than 97% of the NPC occur in endemic region such as Southern China is WHO Type III (Marks *et al.*, 1998).

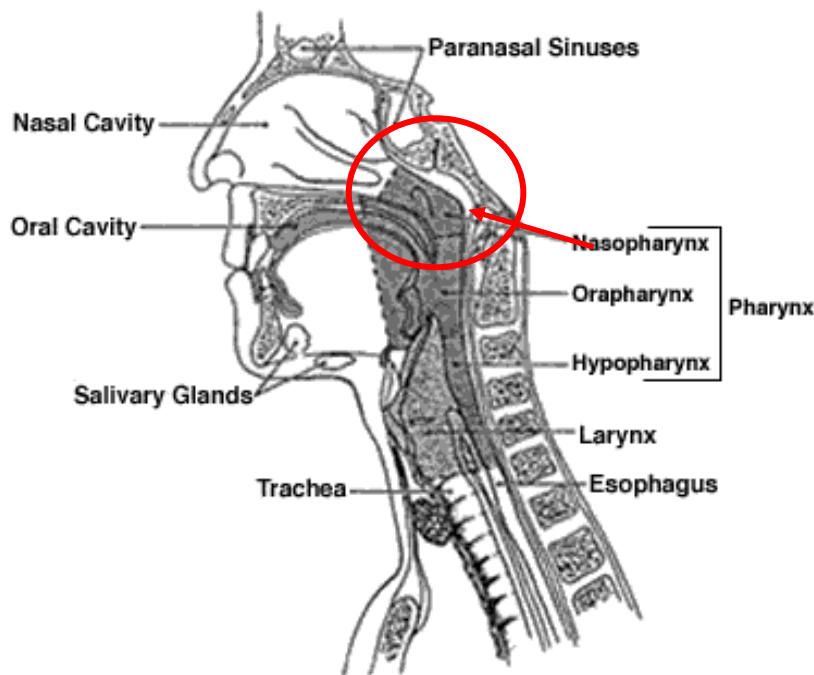


Figure 1: Location of nasopharynx. Nasopharynx is a box-like chamber located at the back of nose toward the base of skull. Taken from American Cancer Society (2006). *Detailed guide: Nasopharyngeal carcinoma*. Retrieved from World Wide Web: <http://www.cancer.org>.

To date, the molecular basis of NPC is still poorly studied. However, it has been suggested that the pathogenesis mechanism of NPC is a complex multistep process. Few epidemiological studies have proposed the involvement of three major aetiological factors in the development of NPC, which includes genetic susceptibility, environmental factors and latent Epstein-Barr Virus (EBV) infection (Tao & Chan, 2007). The ethnic clustering of NPC in Southern China has suggested the strong association between ethnic and genetic influences. Approximately 10 % of NPC incidences are familial cancers (Tao & Chan, 2007). An NPC susceptibility locus has been identified at the human leukocyte antigen (HLA) region. The researchers postulated that some of the HLA antigens have reduced efficiency in combating EBV infection. Prevalent Chinese A2 subtype (HLA-A*0207), instead of prevalent Caucasian

A2 subtype (HLA-A*0201) has been strongly associated with increased NPC risk in a high-resolution genotyping study (Kwok *et al.*, 2004). Moreover, the frequent consumption of salted fish and preserved food as well as the occupational exposure to formaldehyde or wood dust are strongly linked to development of NPC. A study reported that the intake of preserved food contributes to two-fold increment in NPC risk whereas non-preserved food intake leads to 36 % decrement in NPC cases (Tao & Chan, 2007). NPC is very different from other head and neck squamous carcinoma in the fact that it is strongly associated with EBV infection. Most of the NPC patients have higher EBV antibody titers in their serum. In earliest premalignant lesion, latent and clonal viral genomes as well as viral oncoproteins such as latent membrane protein 1 (LMP 1) are detected. This indicated that EBV infection occurred before the clonal expansion of tumour cells. Hence, it is proposed that EBV plays important roles in transforming normal nasopharyngeal epithelial cells into invasive carcinoma.

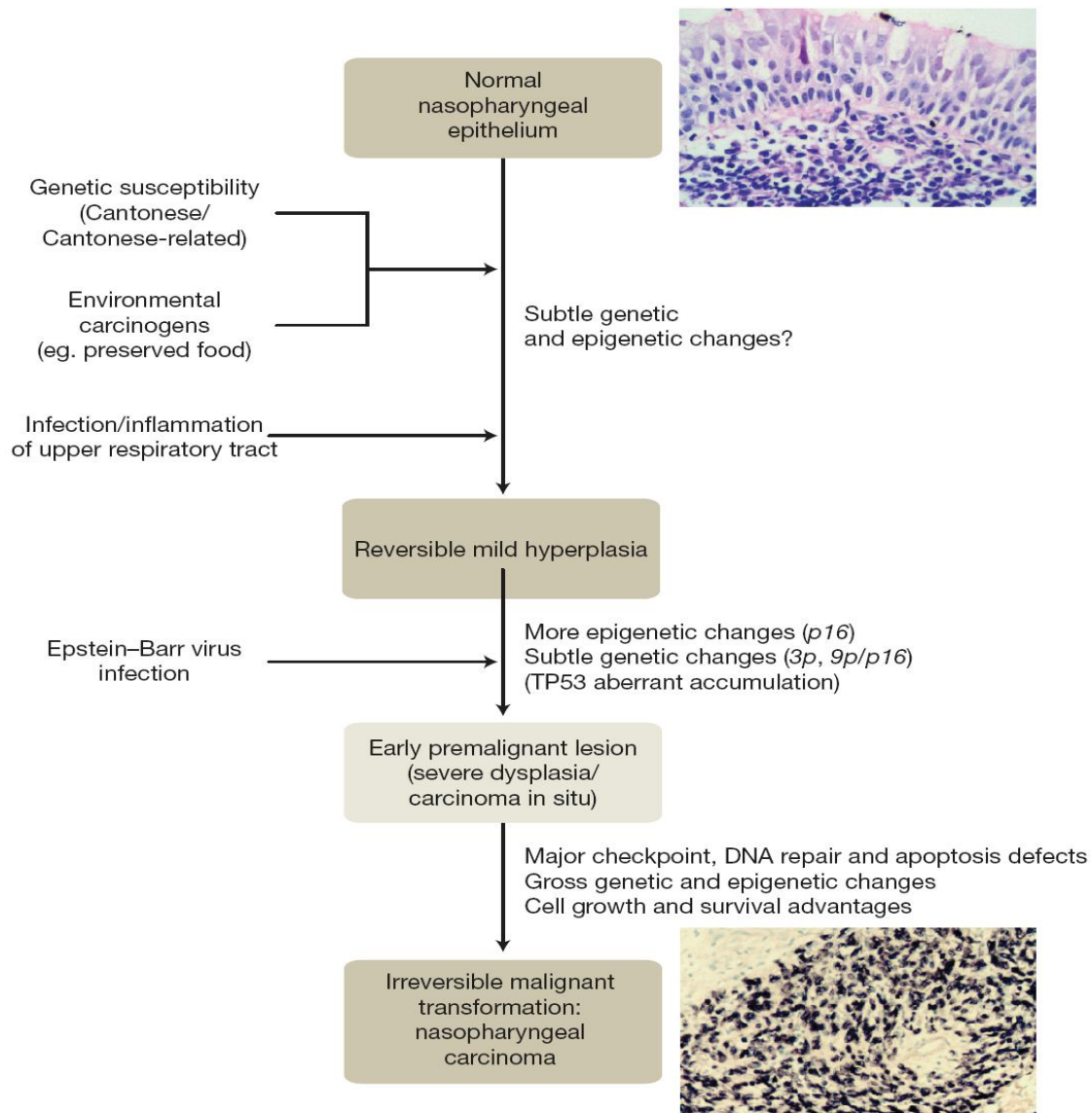


Figure 2: Possible model of nasopharyngeal carcinoma. The whole pathogenesis of NPC might take approximately 40 years. However, the latent EBV infection has accelerated the transition from carcinoma in situ to an NPC case. Taken from Tao & Chan (2007).

Nasopharyngeal carcinoma: molecular pathogenesis and therapeutic developments. *Expert reviews in molecular medicine*, 9 (12).

2.2 Ribosomal Proteins (RPs)

A complete cytogenetic map of 80 structurally different mammalian ribosomal protein genes and four rRNA molecules have been constructed (Uechi *et al.*, 2001). This map consists of information on chromosomal locations of all genes encoding RPs and rRNAs. Thus, this cytogenetic map acts as a useful tool in studying association of RP genes with human disorders. However, the specific functions of each RP still remain unknown.

In order to provide equimolar supply of ribosomal components, RPs are synthesized stoichiometrically, in conjunction with rRNAs (Li *et al.*, 2002). The rRNAs catalyze the protein synthesis while RPs facilitate rRNA folding, protecting them from nuclease degradation as well as manipulating the multistep process of protein synthesis. Some RPs even have substantial extra-ribosomal functions (Wool, 1996).

Most mammalian ribosomal protein genes, like those in prokaryotes, are organized into a small number of operons, with approximately 11 ribosomal proteins under the regulation of a single promoter (Nomura *et al.*, 1984). Indeed, in a eukaryotic genome, RP genes exist as a cluster of a single expressed gene and some inactive, processed pseudogenes throughout the genomes (Kondoh *et al.*, 1992). According to Kenmochi *et al.* (1998), both human sex chromosomes and at least 20 autosomes would have one or more RP genes. This is possible as RP genes are widely dispersed throughout the genome. Since RPs are highly conserved among eukaryotes and prokaryotes during evolution, almost all mammalian RPs

share 40%-80% similarity with yeast RPs (Kenmochi *et al.*, 1998). The sequence studies, together with conservation of ribosomal proteins among eukaryotes have revealed that the human ribosomes are extremely similar with ribosomes in *Drosophila* (Kenmochi *et al.*, 1998). Thus, the effects of mutation in human ribosomal protein genes have been investigated thoroughly in *Drosophila*.

RPS26 and *RPS27* are components of small 40S subunit of ribosome. *RPS26* is a 13.0 kDa protein which belongs to *S26E* family (Human Protein References Database, 2007). The protein is encoded by *RPS26* gene located on chromosome 12q13 (Human Protein References Database, 2007).

According to Vincent *et al.* (1993), *RPS26* gene which is 600 – 700 bp long is highly and constantly expressed in various human adult tissues. Therefore, it could potentially act as an internal control for all experiment involving RNA quantification and gene expression. In recent years, *RPS26* has been widely used as an endogenous control for gene regulation and gene expression study, including expression analysis of Matrilin-2 in human skin (Piecha *et al.*, 2002), differential expression analysis of Gcn512 in mesodermal defects during mouse development and and expression pattern evaluation of homeobox gene in myelomocytic differentiation acute myeloid leukemia.